Supporting information – Fate and Uptake of Pharmaceuticals in Soil – Plant Systems

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SI 1.1 Maximum water holding capacity calculations

Maximum Water Holiding Capacity (MWHC):

Method

Place 50 g of soil in a funnel with a Whatman No.1 filter paper (185mm diameter). Attach a rubber hose with clip closed, add 50 ml water and allow the soil to stand for 30 minutes. After this time, the clip is opened and allowed to drain for 30 minutes, or until it stops draining, and the amount of water drained measured.

Calculations

The difference between the volume of water added and the volume of water drained, minus the water held in the filter paper (7.3 $\pm$ 0.6 (standard deviation) mL), plus the water previously in the soil, is taken as water holding capacity (MWHC).

$$MWHC = 50 - \text{water drained} - 7.3 + \text{water in soil previously.}$$

SI 1.2 Preparation of Ruakura nutrient solution for use during plant growth

General Ruakura method:

The following tables contain the salts used to make the Ruakura solution. The solution was prepared by combining three prepared stock solutions to 1.75 L of deionised water (200 mL of stock B, 200 mL of stock A and 100 mL of the micronutrient supplement) to make a final volume of 2.25 L.

5 mL of Ruakura solution per 250 g of soil[1] was applied twice weekly, (for three weeks), from the day that 50 % germination was counted. After 3 weeks of additions, it was continued with 1 x 5 mL/250 g soil of nutrient solution per week.

Note : Pots were weighed at the start of the experiment (with soil at 60 % MWHC) and watered on a daily basis to maintain this weight. However we were aware that as the plants grow the mass of the pots will change slightly due to an increase in plant biomass. To account for this an additional amount of DI water was added to the pots (1g – 2g) to ensure that the MWHC remained at approximately 60 %.

The nutrient solution was used for the watering purpose on the prescribed one or two days per week after 50% emergence. An additional volume of deionised water was sometimes necessary on nutrient solution watering days to maintain the prescribed weight.

Nutrient Stocks

Macronutrient Stock A (g/L)

Chemical	Weight (g)
$Mg(NO_3)_2.6H_2O$	4.94

$Ca(NO_3)_2.4H_2O$	16.78
NH_4NO_3	8.48
KNO_3	2.28

Macronutrient Stock B (g/L)

Chemical	Weight (g)
KH_2PO_4	2.67
K_2HPO_4	1.64 [or 2.149 g of $K_2HPO_4.3H_2O$]
K_2SO_4	6.62
Na_2SO_4	0.60
NaCl	0.33

Micronutrient Supplement (mg/L)

Chemical	Weight (mg)
H_3BO_3	128.8
$CuCl_2.2H_2O$	4.84
$MnCl_2.4H_2O$	81.1
$(NH_4)6Mo_7O_{24}.4H_2O$	0.83
$ZnCl_2$	23.45
Ferric citrate (Mr 244.9)	592

SI 1.3 Validation of extraction methodologies

Radish, ryegrass and soil were spiked with a known amount of each pharmaceutical and different extraction methods and clean up steps were followed to obtain the highest percentage recoveries. For both soil and plant, 1g of material was extracted. Plants were freeze dried prior to extraction (soil was not) and then either extracted with 2 x methanol (5 mL per extraction), followed by 1 x acetone (5mL) or three extractions of a 70:30 (v/v) acetonitrile and water solution (5 mL per extraction). A comparison between using SPE and no SPE as a clean up step was also made. Results presented below indicate the methods which generated the highest recoveries for the different matrices and thus were adopted in the extraction techniques in this study.

		% Relative recovery					
Matrix	**Extraction**	**Carbamazepine**	**Diclofenac**	**Fluoxetine**	**Propranolol**	**Sulfamethazine**	**Triclosan**
Leaf	ACN:H_20 (SPE)	100.5 ± 4.7	118.7 ± 5.8	89.1 ± 4.3	98.8 ± 4.2	82.4 ± 4.9	117.2 ± 19
Root	ACN:H_20 (SPE)	106.8 ± 4.1	139 ± 10	92.6 ± 5.2	103.9 ± 4.8	75.4 ± 1.7	181.8 ± 48
Soil	MeOH/Acetone	90.42 ± 7.12	85 ± 6.3	68.53 ± 10	109.40 ± 23	91.54 ± 7.54	98.57 ± 4.9

% Relative recovery is the recovery in relation to what was spiked into the matrix prior to extraction

Results show that a ACN:H_20 extraction followed by SPE clean up yielded the best recoveries for radish and ryegrass (both leaf and root) for the range of pharmaceuticals and therefore this method was adopted to analyse plant samples. Best recoveries were obtained for the soil samples using a combination of methanol and acetone extractions.

SI 1.4 LC-MS/MS parameters used for the analysis of the compounds

Compound	Parent ion (m/z)	MRM product ions (m/z)	Collision energy (V)	Retention time (min)
Propranolol	260 $[M+H]^+$	116 183	25 25	5.25
Propranolol-D_7	267 $[M+H]^+$	116 188	25 25	5.23
Sulfamethazine	279 $[M+H]^+$	92 124	35 35	5.09
Carbamazepine	237 $[M+H]^+$	193 194	40 30	5.41
Carbamazepine-D_{10}	247 $[M+H]^+$	202 204	40 30	5.44
Fluoxetine	310 $[M+H]^+$	44 148	20 20	5.33
Fluoxetine-D_5	315 $[M+H]^+$	44 156	20 20	5.38
Diclofenac	294 $[M-H]^+$	214 250	20 20	5.97
Diclofenac-D_4	298 $[M-H]^+$	217 254	20 20	5.93
Triclosan	287 $[M-H]^+$ 289 $[M-H]^{+a}$	287 289	2 2	6.09
Triclosan-D_3	290 $[M-H]^+$ 292 $[M-H]^{+a}$	290 292	2 2	6.16

[a] ^{37}Cl isotope of TCS and TCS-d_3

SI 1.4.1 Analytical lower limits of quantification (LOQs) for the LC-MS/MS method used and within the plant and soil matrices in µg/L and µg/g (dry weight).

Compound	LC-MS/MS	Soil (µg/L)	Soil (µg/g)	Ryegrass leaf (µg/L)	Ryegrass leaf (µg/g)	Radish leaf (µg/L)	Radish leaf (µg/g)	Radish bulb (µg/L)	Radish bulb (µg/g)
Propranolol	1	2.6	0.003	5.3	0.005	4.4	0.004	5.9	0.006
Sulfamethazine	0.5	0.6	0.0006	4.6	0.005	6.7	0.007	10	0.010
Carbamazepine	0.5	0.7	0.0007	4.2	0.004	5.0	0.005	2.6	0.003
Fluoxetine	2.5	7.1	0.007	7.1	0.007	8.1	0.008	6.6	0.007
Diclofenac	2.5	2.7	0.003	2.1	0.002	3.0	0.003	4	0.004
Triclosan	5	17.9	0.018	11.1	0.011	7.3	0.007	6.3	0.006

SI 1.5 Soil and pore water dissipation: model parameters

Statistical indices for single first order (SFO), first order multi-compartment models[2] (FOMC) or bi-exponential models (BFO) using to model the degradation rates of the pharmaceuticals in the soil and pore water.

Soil:

Pharmaceutical	Model	DT_{50}	DT_{90}	SSRes	RMSE	χ^2 (tabulated χ^2)	Model error	Rate constant (k1)or (α/β)	r^2
Carbamazepine	*	> 40 d	> 40 d						
Diclofenac	SFO	0.50	1.64	0.51	13.68	0.084 (9.49)	29.59	(1.4)	0.99
Fluoxetine	*	> 40 d	> 40 d						
Propranolol	*	> 40 d	> 40 d						
Sulfamethazine	SFO	0.99	3.29	119.16	67.16	3.63 (9.48)	223.67	(0.7)	0.99
Triclosan	SFO	11.55	38.38	195.66	37.36	3.36 (9.49)	152.78	(0.06)	0.97

* No significant difference between 0 d and 40 d measured concentrations therefore data was not modelled to determine degradation rates

Pore water:

Pharmaceutical	Model	DT50	DT90	SSRes	RMSE	χ^2 (tabulated χ^2)	Model error	Rate constant (k_1/k_2,C01/C02,α/β)	r^2
Carbamazepine	*	> 40 d	> 40 d						
Diclofenac	FOMC	19.65	2.57E+03	61.47	8.75	7.58 (7.81)	40.81	(α = 0.79, β = 0.34)	0.88
Fluoxetine	*	> 40 d	> 40 d						
Propranolol	*	> 40 d	> 40 d						
Sulfamethazine	BFO	-	-	45.43	0.12	5.94 (5.99)	6.65	C01 = 91, C02 = 9, k_1 =0.85, k_2 = 0.017)	0.99
Triclosan	*	> 40 d	> 40 d						

* No significant difference between 0 d and 40 d measured concentrations therefore data was not modelled to determine degradation rates

SI 1.5.1 Equation for DT50/DT90

For BFO models no solution exists.

Time for 50 % or 90 % decrease in chemical concentration can be modelled for the SFO using the rate constant (k):

$$DT50 = \ln 2/k$$

$$DT90 = \ln 10/k$$

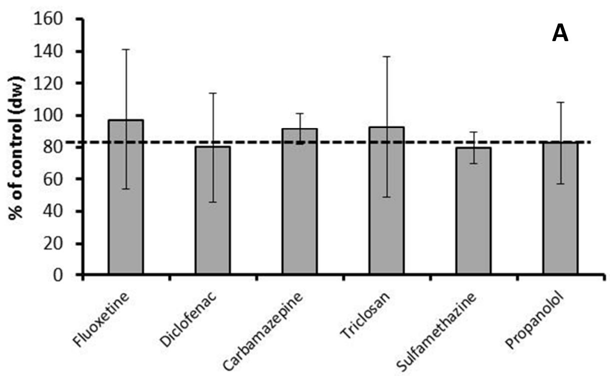
For results using the FMOC model:

$$DT50 = \beta*(2^{(1/\alpha)}-1)$$

$$DT90 = \beta*(10^{(1/\alpha)}-1)$$

SI 1.6 Effect of pharmaceutical treatment on plant growth

Figure 1 Percentage growth of control for radish leaf (A) and bulb (B) as a result of pharmaceutical treatment (fluoxetine, diclofenac, carbamazepine, triclosan, sulfamethazine and propranolol). Average value provided with error bars representing ± standard deviation, based on dry weight of plant material.



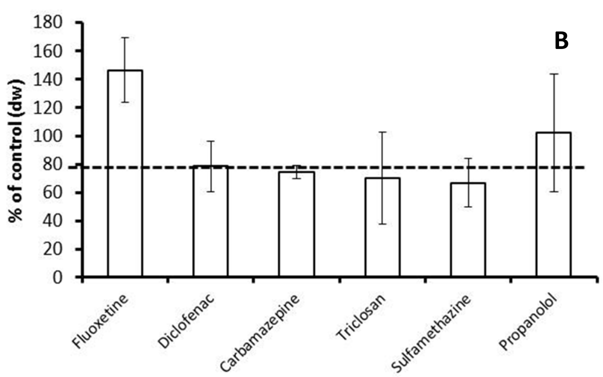
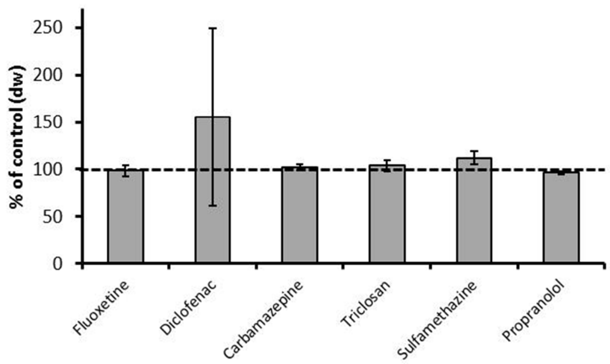


Figure 2 Percentage growth of control for ryegrass as a result of pharmaceutical treatment (fluoxetine, diclofenac, carbamazepine, triclosan, sulfamethazine and propranolol). Average value provided with error bars representing ± standard deviation, based on dry weight of plant material.



SI 1.7 Human exposure calculations

The human risk of consumption from crops grown in pharmaceutically contaminated soil was calculated. Calculations were based on DEFRA statistics[3] which estimate that in the United Kingdom an adult (70 kg) consumes 395.5 g of above ground crops and 159 g of below ground crops per day. Therefore ryegrass was assumed representative of an above ground crop species and radish bulb was representative of a below ground crop species. Acceptable daily intakes were based on the minimum therapeutic dose (mg/person/day) with a safety factor of 100 applied. Using calculated UF_{soil} and measured soil concentrations ([a] = Duran-Alverez et al., 2009; [b] = Dalkmann et al.,2012; [c] = Vazquez-Roig et al.,2012)[4–6] we could estimate realistic crop concentrations and thus how much would be in a human diet. A percentage of the ADI for each pharmaceutical was then calculated for each pharmaceutical. As sulfamethazine uptake was below LOQ this was removed from the analysis.

Ryegrass - assumed representative above ground crop

	Soil conc. (mg/kg)	UF soil	Plant conc. (mg/kg)	Plant conc. (mg/g)	Consumption per person (g/day)	Conc. In 359.5 g of crop (mg/day)	Min. therapeutic dose (mg/person/day)	ADI (mg/person/day)	% of ADI in 359.5 g crop
CBZ	0.0065[a]	65.26	0.42	0.00042	359.5	0.1525	400	4	**3.81**
DCF	0.0005[b]	6.82	0.0037	3.68E-06	359.5	0.0013	75	0.75	**0.18**
FLX	0.0067[b]	0.08	0.0005	5.11E-07	359.5	0.0002	20	0.2	**0.09**
PRL	0.0004[c]	11.04	0.0044	4.42E-06	359.5	0.0016	80	0.8	**0.20**
TCS	0.0186[a]	37.59	0.70	0.00070	359.5	0.2514	30	0.3	**83.8**

Radish - assumed representative of below ground crop

	Soil conc. (mg/kg)	UF soil	Plant conc. (mg/kg)	Plant conc. (mg/g)	Consumption per person (g/day)	Conc. In 159 g of crop (mg/day)	Min. therapeutic dose (mg/person/day)	ADI (mg/person/day)	% of ADI in 159 g crop
CBZ	0.0065[a]	8.28	0.05	5.38E-05	159	0.00856	400	4	**0.21**
DCF	0.0005[b]	5.39	0.00	2.91E-06	159	0.00046	75	0.75	**0.06**
FLX	0.0067[b]	0.36	0.00	2.43E-06	159	0.00039	20	0.2	**0.19**
PRL	0.0004[c]	1.20	0.00	4.79E-07	159	0.00008	80	0.8	**0.01**
TCS	0.019[a]	0.12	0.00	2.26E-06	159	0.00036	30	0.3	**0.12**

References:

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